메틸글루코시드 아크릴레이트/메타크릴레이트의 효소적 합성

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Lipase-catalyzed synthesis of β-methylglucoside acrylate/methacrylate

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Introduction

Sugars are particularly interesting group of polyfunctional compounds. They are biologically relevant and contain multiple hydroxyl groups which are nearly indistinguishable chemically, yet differentiable enzymatically. Their combination into polymers is a natural event, leading to a various field of polysaccharides. Sugars have also been modified enzymatically and incorporated into polymers quite distinct from polysaccharides. Thereby, the esterification of sugars may have considerable impact in the synthesis of food intergredients, surfactants, sweeteners, and biochemical and pharmaceutical intermediates.

Sugar esters, which are produced by esterification of sugar donor with acyl donor, are biocompatible, biodegradable, non-ionic and non-toxic, and can be synthesized from renewable sources. The specific properties of sugar ester depend on the acyl donor, the sugar moiety, the degree of substitution, and its chemical structure. In particular, sugar esters have been reported in the field of biomaterial and optical material [1, 2].

For the past few years, several researchers have investigated the lipase-catalyzed synthesis of sugar-containing acrylic esters due to their biomedical applicability [3, 4]. Acrylic acid has a high hydrophilic characteristic due to its carboxylic group capable of esterifying with the hydroxyl group of sugars. Moreover, it has a vinyl group that can be polymerized. Vinyl acrylate has also been shown to act as irreversible acylation agent [5], and to be contained property of acrylic acid. Therefore, acrylic esters are expected to be efficient monomers for sugar polymers having high hydrophilicity and biocompatibility.

In this work, the enzymatic esterifications of acrylic acid/vinyl methacrylate with β-methylglucoside were carried out by Novozym 435, lipase from *Candida antarctica*, as a biocatalyst. Optimal reaction conditions for the esterificatin of β-methylglucoside and acrylic acid/vinyl methacrylate such as reaction media, enzyme amount, molar ratio of substrates, initial substrate concentration and reaction temperature were determined.

Experimental

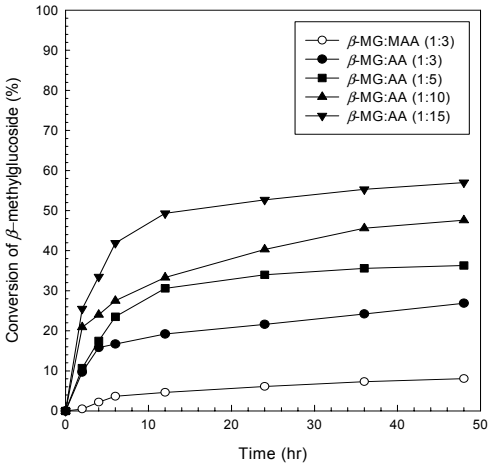
Esterification reaction was carried out in screw capped test tubes. β-Methylglucoside prepared in specific concentrations was added in 10 mL *t*-butanol. Reactions were performed by adding Novozym 435 with magnetic stirring. The enzyme concentrations were 1, 3 and 5 % (w/v), and as were the cases for the molar ratios of β-methylglucoside:acrylic acid (vinyl methacrylate) were 1:3, 1:5, 1:10 and 1:15 (1:1, 1:2 and 1:3). The initial concentrations of β-methylglucoside and reaction temperatures were varied in the range of 30 ~ 60 g/L and 45 ~ 60 ℃, respectively. Reactions were monitored by HPLC using a carbohydrate column

(Nova-Pak, 250 × 4.6 mm, Waters) with detection by RI. For the quantification for the β-methylglucoside, the eluant was acetonitrile/water (80:20, v/v) at the flow rate of 1.2 ml/min.

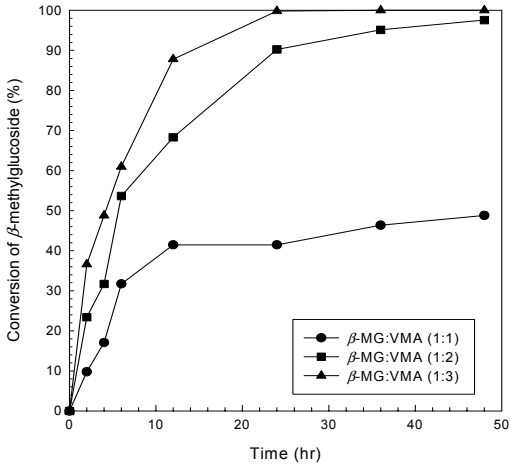
Results and Discussion

Optimal molar ratio

The degree of esterification increased with the overall range of the molar ratio of β-methylglucoside and acrylic acid, and the maximum conversions at 1:3, 1:5, 1:10 and 1:15 after 48 h were 26, 35, 45 and 55 %, respectively (Fig. 1. a). In addition, conversion of β-methylglucoside with methacrylic acid at 1:3 molar ratio reached 8 % after 48 h. In the presence of excess vinyl methacrylate (Fig. 1. b), however, the highest yield was obtained at a molar ratio of β-methylglucoside to vinyl methacrylate of 1:3, the conversion of β-methylglucoside reached 100 % at 24 h. In this reaction, the vinyl alcohol leaving group tautomerized to acetaldehyde, thereby making the reactions irreversible. Although acetaldehyde is known to deactivate many enzymes, the lipase from *Candida antarctica* seemed not to be affected. Thereby, vinyl methacrylate was exchanged for methacrylic acid in subsequent esterifications. Moreover, increasing molar ratio should have promoted a shift of the reaction equilibrium towards formation of the ester. However, the higher the molar ratio, the higher the residual concentration of acrylic acid and vinyl methacrylate. It could be cause of expensive steps for sugar ester purification. Therefore, not only 1:15 molar ratio of β-methylglucoside to acrylic acid but also 1:3 molar ratio of β-methylglucoside to vinyl methacrylate was used in subsequent experiments.



(a) molar ratio of β-methylglucoside/acrylic acid



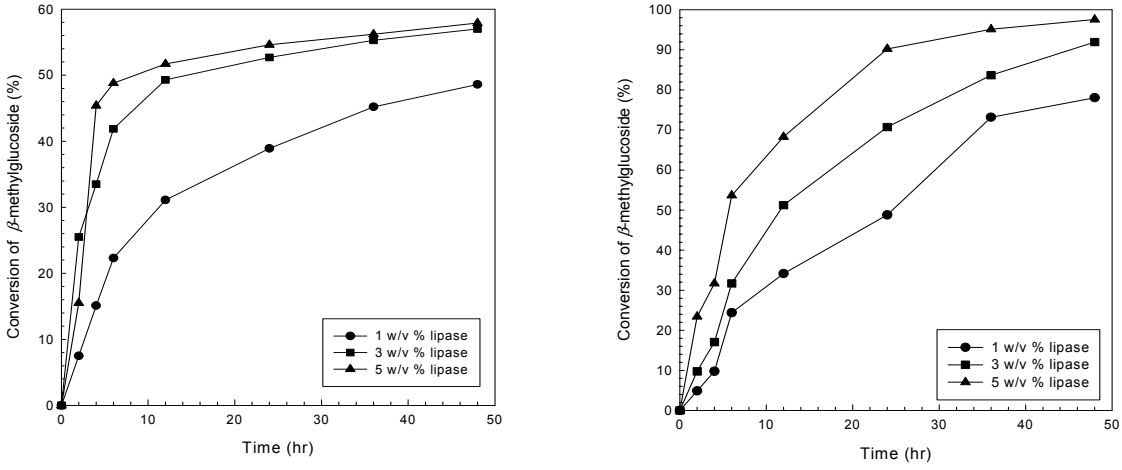
(b) molar ratio of β-methylglucoside/vinyl methacrylate

Fig. 1. Effect of molar ratio on the synthesis of β-methylglucoside acrylate/methacrylate

Optimal concentration of enzyme

For the biocatalystic process to be more competitive than chemical process, the amount of enzyme used becomes an important economic factor. For this reason, a compromise between productivity and enzyme content should be achieved. Fig. 2 shows the conversions of β-methylglucoside in the range of 1~5 % (w/v) enzyme. The best conversion was obtained

with 5 % (w/v) enzyme. However, there was not much difference in the maximum value between 3 and 5 % (w/v) enzyme on the synthesis of β-methylglucoside acrylate as well as β-methylglucoside methacrylate. Although higher conversion would be expected at a higher enzyme concentration, it seemed to be no economics in the viewpoint of cost-reduction. Thereby the amount of enzyme over 5 % (w/v) was not used.



(a) acrylic acid (b) vinyl methacrylate

Fig. 2. Effect of enzyme concentration on the esterification of β-methylglucoside

Optimal reaction temperature

The reaction temperature had a great influence on the rate of esterification. The effect of temperature on sugar ester formation from β-methylglucoside by lipase Novozym 435 is illustrated in Table 1. The optimal temperature of esterification with acrylic acid was observed at 50 ℃, and subsequently the reaction rate reduced over at 55 ℃. *Candida antarctica* lipase had a good thermoresistance in *t*-butanol, and Novozym 435 was known as heat-tolerant enzyme which maintains its activity even at 90 ℃. Therefore it was considered that the decrease in reaction rate was caused not by rising temperature but by increasing water activity at higher temperature. Water activity could influence the reaction rate as well as the enzyme activity in organic media. The water transfer was determined by Fick's first law which was proportional to the diffusion coefficient for water and used to describe the effects of water activity control in esterification. The diffusion coefficient was temperature dependent parameter, and the increase in diffusion coefficient and water transfer by rising temperature consequently caused the water activity in reaction system to increase. Therefore high water activity due to increasing temperature was considered to be the major factor to decrease the reaction rate.

The conversion of β-methylglucoside with vinyl methacrylate was found to increase when the reaction temperature was increased. However, the upper temperature limit was fixed at 60 ℃ to avoid denaturation of enzyme as well as to protect browning reaction of β-methylglucoside. Although both conversions and initial rates at 55 and 60 ℃ were observed more slightly increase than those at 50 ℃, the conversions of β-methylglucoside at 50, 55 and 60 ℃ reached all 100 % after 24 h. At the same conversion, the lower the reaction temperature, the lower the operation cost on the production process of sugar ester. For this reason, subsequent experiments were carried out at 50 ℃.

Table 1. Selection of temperature on sugar ester formation

Temperature (℃)	Conversion (%)			
	Acrylic acid (48 h)	Vinyl methacrylate		
		12 h	24 h	36 h
45	55.6	90.2	97.6	100
50	57.9	87.8	99.8	100
55	51.9	95.1	100	100
60	43.0	95.1	100	100

Initial concentration of β-methylglucoside

The final β-methylglucoside acrylate/methacrylate quantity obviously depends on the amount of β-methylglucoside that can be converted by the lipase. Within the framework of process optimization, it is important to study the effect of the β-methylglucoside concentration on initial rates and yields. Table 2 shows that initial rates are proportional to β-methylglucoside concentrations from 30 to 60 g/l. The conversion of β-methylglucoside with acrylic acid after 48 h incubation reached the maximum value at 40 g/l, and the highest conversion of β-methylglucoside with vinyl methacrylate was observed at 50 g/l after 12 h. However, the conversion with acrylic acid (or vinyl methacrylate) at 40 g/l (50 g/l) was slightly higher than that at 50 g/l (60 g/l), and the use of 50 g/l (60 g/l) would seem to be more effective compared with 40 g/l (50 g/l) since a higher concentration of substrate with similar conversion rate was expected to produce more amounts of products.

Table 2. Influence of the initial concentration of β-methylglucoside on esterification

Initial concentration of β-methylglucoside (g/l)	Conversion (%)			
	Acrylic acid (48 h)	Vinyl methacrylate		
		12 h	24 h	36 h
30	49.2	83.9	96.8	100
40	57.9	87.8	99.8	100
50	57.1	98.0	100	100
60	48.9	96.8	100	100

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